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Additional Restriction Endonuclease Cleavage Sites on the Bacteriophage P22 Genome

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We present complete restriction endonuclease cleavage site maps of the bacteriophage P22 chromosome for 16 enzymes with six base recognition sequences, thereby positioning 116 new sites on the chromosome. Twenty-four such restriction maps for P22 DNA, containing 162 sites, have now been completed, and three enzymes were found that did not cut P22 DNA. Our results are consistent with the ideas that *Cla*I does not cleave the methylated recognition sequence ATCGA^{me}T or A^{me}TCGAT and *Stu*I does not cleave the methylated recognition sequence AGGCC^{me}T.

A large number of restriction endonuclease sites have been mapped on the bacteriophage P22 chromosome. Maps of the cleavage sites for *Eco*RI (9), *Hin*dIII (4), *Bam*HI (G. Weinstock, Ph.D. thesis, Massachusetts Institute of Technology, Boston, 1977), *Sma*I, *Xho*I, *Bgl*I, *Sal*I, *Sst*I (3), and *Pst*I (15) have been determined. In addition, it is known that *Kpn*I and *Bgl*II do not cleave P22 DNA (3). Knowledge of the physical location of these sites has been extremely valuable in the study of the structure and organization as well as the expression of the P22 chromosome (3, 15). It has also been most useful in the study of the packaging of the chromosome during phage assembly (2, 7, 8, 10). In the course of our work with phage P22 we have determined the locations of the sites at which a number of additional restriction enzymes cleave its DNA. We present them here as additional physical markers on the chromosome of phage P22 in the belief that they will be useful in the future study of this phage.

P22 DNA isolated from virus particles was cleaved with *Acc*I, *Ava*I, *Bal*I, *Bst*EII, *Cla*I, *Eco*RV, *Hpa*I, *Nae*I, *Pvu*I, *Pvu*II, *Sph*I, *Sst*II, *Stu*I, *Tth*111I, *Xba*I, and *Xmn*I (data not shown). The fragments were separated by electrophoresis in agarose (2, 9) or acrylamide gels (2), and their sizes were determined by comparison with *Eco*RI digests of phage P22 or lambda DNA (9) or various restriction enzyme digests of plasmid pBR322 (17). These fragment sizes are shown in Table 1. In each case, as has been done with the previously determined maps (3, 15), the fragments were named alphabetically by size, with the largest fragment being called A. It should be noted that the leftmost cleavage (Fig. 1) in each case gives rise to a "pac fragment," which has a restriction cut at its right end and a packaging cut at its left end (2, 3, 7, 8). These pac fragments are not included in Table 1, although they were seen in submolar amounts in the digests. Since P22 DNA is circularly permuted (17, 18), only a fraction of the phage chromosomes give rise to a pac fragment (7) (e.g., *Cla*I cleavage of permuted mature molecules yields fragment H with ends at *Cla*I sites 1 and 11, and a *Cla*I pac fragment with ends near *pac* and at *Cla*I site 1).

The cleavage sites of these 16 enzymes were mapped relative to those previously located on the genome (3, 15) by analyzing various digests of P22 DNA containing the enzyme whose cleavage sites were under investigation and one whose cleavage sites were already known. In all cases the sites were located within cloned fragments of P22 DNA (3, 13, 15). The only exceptions to this were some sites lying between *Pvu*II site 2 (map position 0.755) and *Hin*dIII site 10 (map position 0.832). This region of the P22 genome was not present on the cloned fragments available in Utah during this work, so *Cla*I, *Eco*RV, *Nae*I, *Stu*I, and *Xmn*I sites within this region were determined purely from the analysis of phage DNA. When possible, the locations of sites were confirmed on whole phage DNA. *Acc*I sites 4, 5, 6, 7, 8, and 9 were located by comparison with the base sequence of that region (R. Sauer, W. Krovatin, A. Poteete, and P. Berget, Biochemistry, in press), and all sites between map positions 0.699 and 0.747 were

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TABLE 1. Phage P22 DNA restriction fragment sizes

Restriction endonuclease[a]	Fragment	Size (bp)[b]	Restriction endonuclease	Fragment	Size
*Acc*I	A	10,610	*Hpa*I	A	9,900
	B	8,400		B	4,450
	C	7,780		C	4,330
	D	4,920		D	3,450
	E	4,200		E	3,370
	F	3,370		F	3,130
	G	720		G	3,040
	H	580		H	2,750
	I	440		I	1,910
	J	245		J	1,400
	K	185		K	1,250
	L	105		L	1,000
	M	75		M	920
				N	260
*Ava*I	A	10,820		O	240
	B	9,030			
	C	5,770	*Nae*I	A	15,800
	D	3,830		B	12,100
	E	3,330		C	8,990
	F	2,990		D	2,560
	G	2,160		E	1,200
	H	1,830		F	930
	I	1,460			
	J	200	*Pvu*I	A	23,170
	K	190		B	9,940
				C	4,330
*Bst*EII	A	31,530		D	4,160
	B	9,150			
	C	940	*Pvu*II	A	30,040
				B	11,560
*Cla*I	A	7,990			
	B	5,370	*Sst*II	A	22,010
	C	5,240		B	16,470
	D	4,740		C	3,120
	E	4,500			
	F	4,460	*Stu*I	A	9,670
	G	3,540		B	8,900
	H	3,290		C	7,820
	I	1,410		D	5,700
	J	1,000		E	5,410
	K	60		F	2,500
				G	1,580
*Eco*RV	A	7,860			
	B	7,280	*Tth*111I	A	14,980
	C	5,280		B	12,980
	D	4,370		C	6,700
	E	3,000		D	5,030
	F	2,250		E	1,900
	G	2,080			
	H	1,960	*Xmn*I	A	6,280
	I	1,460		B	5,490
	J	1,300		C	5,450
	K	1,300		D	5,120
	L	1,000		E	3,990
	M	680		F	3,040
	N	560		G	2,790
	O	420		H	2,290
	P	330		I	1,750
	Q	310		J	1,210
*Xmn*I	K	1,160	*Xmn*I	O	500
	L	1,040		P	300
	M	850		Q	150
	N	620			

[a] Restriction endonucleases were purchased from New England Biolabs, Bethesda Research Laboratories, Miles Laboratories, and Boehringer-Mannheim and were usually used under the conditions suggested by the manufacturer. Most double digests were performed in TA buffer (66 mM potassium acetate, 33 mM Tris-acetate [pH 7.9], 10 mM magnesium acetate, 10 mM dithiothreitol). Phage and plasmid DNAs were isolated as previously described (2, 3, 15).

[b] The large (more than 3,000 base pair [bp]) fragment sizes shown here are those derived from the cleavage site locations given in Table 2. In no case was there a significant difference between the values determined this way and those simply measured directly.

TABLE 2. Location of restriction endonuclease cleavage sites on phage P22 DNA

Restriction endonuclease	Cleavage site	Map position	Restriction endonuclease	Cleavage site	Map position
*Acc*I	1	0.060	*Cla*I	1	0.058
	2	0.178		2	0.060
	3	0.184		3	0.084
	4	0.385		4	0.198
	5	0.387		5	0.390
	6	0.401		6	0.498
	7	0.404		7	0.605
	8	0.421		8	0.690
	9	0.426		9	0.730
	10	0.436		10	0.850
	11	0.517		11	0.979
	12	0.618			
	13	0.873	*Eco*RV	1	0.040
				2	0.075
*Ava*I	1	0.006		3	0.264
	2	0.011		4	0.295
	3	0.228		5	0.326
	4	0.308		6	0.336
	5	0.568		7	0.349
	6	0.640		8	0.365
	7	0.692		9	0.540
	8	0.697		10	0.587
	9	0.831		11	0.611
	10	0.923		12	0.716
	11	0.964		13	0.794
				14	0.848
*Bal*I	1	0.164		15	0.855
				16	0.982
*Bst*EII	1	0.010		17	0.990
	2	0.032	*Hpa*I	1	0.049
	3	0.252		2	0.130

TABLE 2—*Continued*

Restriction endonuclease	Cleavage site	Map position	Restriction endonuclease	Cleavage site	Map position
*Hpa*I	3	0.136		4	0.879
	4	0.219		5	0.901
	5	0.326		6	0.930
	6	0.356			
	7	0.429	*Pvu*I	1	0.286
	8	0.667		2	0.390
	9	0.713		3	0.490
	10	0.779		4	0.729
	11	0.854			
	12	0.880	*Pvu*II	1	0.477
	13	0.886		2	0.755
	14	0.920			
	15	0.945	*Sph*I	None	
*Nae*I	1	0.310	*Sst*II	1	0.205
	2	0.526		2	0.280
	3	0.817		3	0.809

TABLE 2—*Continued*

Restriction endonuclease	Cleavage site	Map position	Restriction endonuclease	Cleavage site	Map position
*Stu*I	1	0.094	*Xmn*I	1	0.065
	2	0.327		2	0.216
	3	0.541		3	0.339
	4	0.729		4	0.471
	5	0.767		5	0.496
	6	0.827		6	0.508
	7	0.957		7	0.581
				8	0.595
				9	0.650
*Tth*111I	1	0.083		10	0.678
	2	0.204		11	0.707
	3	0.365		12	0.796
	4	0.411		13	0.835
	5	0.723		14	0.856
				15	0.987
				16	0.990
*Xba*I	1	0.421		17	0.998

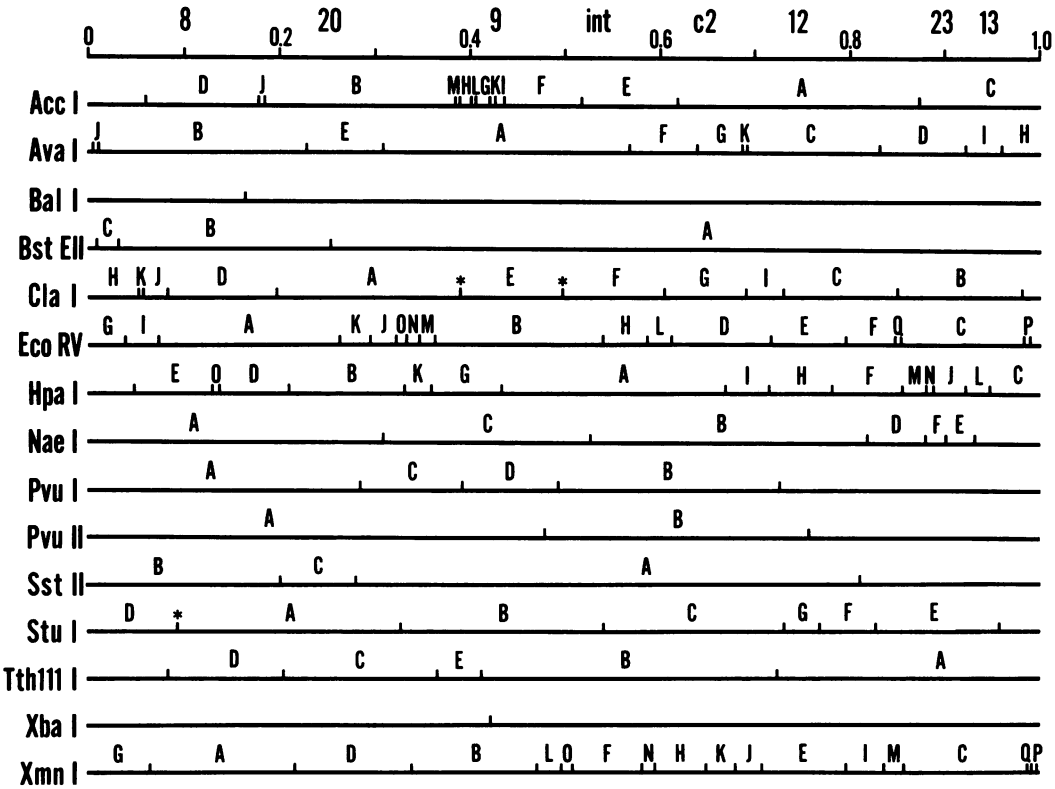

FIG. 1. Restriction maps of the phage P22 genome. The restriction endonuclease cleavage sites (Table 2) are plotted on the physical map as described by Chisholm et al. (3) and Rutila and Jackson (15). The circular map is arbitrarily opened at a point defined to be 400 base pairs to the left of *Bst*EII site 1, which coincides with the DNA packaging initiation site (2, 7, 10). The top line indicates distance as a fraction of the complete genome (about 41,600 base pairs). The positions of several genes are given at the top for orientation (15). The asterisks (*) denote sites which are incompletely cut in phage DNA (see the text). Restriction sites for each enzyme are named 1, 2, 3, etc., from left to right on each map (see Table 2).

confirmed in the base sequence of that region (N. Franklin, personal communication). The locations of the sites (data not shown) thus determined are given in Table 2 and shown graphically in Fig. 1. There is some uncertainty in these locations, and since we could not perform all possible double digests, nearby sites (within about 0.005 genome lengths; e.g., *Hpa*I site 11 and *Eco*RV site 15) have not necessarily been ordered with respect to each other.

During this work we observed that *Cla*I sites 5 and 6 and *Stu*I site 1 were incompletely cut on phage DNA and not cut at all when present in plasmid clones. The partial cleavage of *Cla*I site 5 on P22 DNA was independently observed and similarly interpreted by Sauer et al. (in press). The *Cla*I recognition sequence ATCGAT (14) can overlap the sequence methylated by the *dam* methylase GA^{me}TC (1, 5). Similarly, the *Stu*I recognition sequence AGGCCT (16) can overlap the *dcm* methylation site CC^{me}TGG (11). We believe that these observations can be adequately explained if the *Salmonella typhimurium* strain (DB7000) (19) in which our phage were grown does not completely methylate the *dam* and *dcm* methylation sites on P22 phage DNA during infection. It is thought that these sites are normally completely methylated in the *S. typhimurium* chromosome (6) and in plasmids carried in *dam*$^+$ *dcm*$^+$ strains of *Escherichia coli*. (Our cloned fragments were propagated in such an *E. coli* [3, 15].) A final requirement for this explanation is that *Cla*I and *Stu*I endonucleases actually be unable to cleave sites methylated as indicated above, and that these three sites actually do overlap a methylase recognition sequence. The known base sequence shows that *Cla*I site 5 does in fact overlap a *dam* methylase site (Sauer et al., in press) and should be methylated as follows:

5′-TATCGA^{me} T C-
3′-ATAGCT A^{me}G-

Mayer et al. (12) made a similar interpretation of similar observations of *Cla*I digests of phage lambda DNA, and our inspection of the phage lambda DNA sequence (F. Sanger, personal communication) supports their conclusions. However, the conclusion that *dcm*-mediated cytosine methylation of the *Stu*I recognition site may block cleavage has not been made before. The fact that phage lambda and phage P22 DNAs appear to be incompletely methylated is not yet understood; DNA replication and packaging may be too rapid for the methylase activity present, or phage infection might partially block methylation.

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